POSTER SESSION

1122

Vascular Signalling: Implication for Atherosclerosis

Tuesday, March 09, 2004, 9:00 a.m.-11:00 a.m.
Morial Convention Center, Hall G
Presentation Hour: 9:00 a.m.-10:00 a.m.

1122-165 Dimerization of Mitogen-Activated Protein Kinase and Auto-Phosphorylation of Akt Occur in the Newly Differentiated Endothelial-Like Cells From Adult Bone Marrow Stem Cells In Vitro

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The present experiments were conducted to investigate the differences of biological behavior and signal transduction pathways between the newly differentiated endothelial cells and normal mature endothelial cells. Cultured mouse adult bone marrow multipotent progenitor cells (MAPCs) were used as the source of stem cells in this study. The MAPCs were induced to differentiate into endothelial cells in serum-free medium in the presence of vascular endothelial growth factor. The course of stem cell differentiation into endothelial cells was monitored with endothelial cell specific markers including Von Willebrand factor (vWF). The differentiating cells started to express vWF 10 days after initiation of differentiation. At day 14 of cell differentiation, it was found that the newly-differentiated endothelial-like cells derived from MAPCs formed much denser networks of branching and anastomosing cords on growth factor reduced Matrigel than did normal mature endothelial cells. It was also observed that the newly-differentiated endothelial cells had much greater growth potential than did normal mature endothelial cells. Phosphorylated mitogen-activated protein kinase (MAPK) dimers of 84 Kd were identified in the newly differentiated endothelial cells without stimulation. Phosphorylation of the serine/threonin protein kinase Akt was also observed in these unstimulated newly differentiated cells. Neither the MAPK dimers nor auto-phosphorylation of Akt was found to be present in normal cultured mature endothelial cells. These results suggest that both MAPK and Akt are constitutively activated in the newly differentiated endothelial cells. The presence of phosphorylated MAPK dimers and constitutive phophorylation of Akt in the newly differentiated endothelial-like cells may contribute to their unique biological behaviors.

1122-166 Marked Upregulation of Lipoxygenase-1, a Receptor for Ox-Low-Density Lipoprotein in Atherosclerosis, and Its Total Ablation by Candesartan and Rosuvastatin Given Concurrently

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Background: LOX-1 is a receptor for ox-LDL, which is upregulated in atherosclerosis. Recent studies show its upregulation by ox-LDL and angiotensin II type 1 (AT1) receptor activation.We postulated that control of dyslipidemia with rosuvastatin, an HMG CoA reductase inhibitor, blockade of AT1 activation with candesartan, would have a synergistic inhibitory effect on LOX-1 expression and evolution of atherosclerosis.
Methods and Results: Apo-E knockout mice were fed high-cholesterol diet (1% cholesterol) alone, or with candesartan (1mg/kg/d) or rosuvastatin (1mg/kg/d) or both. Twelve weeks later, the extent of atherosclerosis was determined by Sudan IV staining. Apo-E knockout mice with high-cholesterol diet had extensive atherosclerosis. Candesartan and rosuvastatin each decreased the extent of atherosclerosis ($P<0.02$, n=5 each group) independent of reduction in total- and LDL-cholesterol. However, the combined feeding of candesartan and rosuvastatin reduced atherosclerosis in a synergistic fashion ($P<0.01$ vs., control and $P<0.05$ vs. candesartan and rosuvastatin alone). The expression of LOX-1 was upregulated (>10-fold) by high-cholesterol diet in apo-E knockout mice (col. 2 v. 1, figure below). While candesartan and rosuvastatin (col. 3 and 4) each had a small inhibitory effect on the expression of LOX-1, the combination therapy (col. 5) ablated the expression of LOX-1 below the levels seen in normocholesterolemic C57BL/6J mice (background for apo-E knockout mice). Combination of candesartan and rosuvastatin also ablated the expression of NF-κB and p38 MAPK, whereas candesartan and rosuvastatin alone had only a modest effect.
Conclusion: This study, for the first time, demonstrates that the combination of candesartan and rosuvastatin markedly affects the expression of p38 MAPK, redox-sensitive NF-κB and LOX-1. These alterations then lead to a marked anti-atherosclerotic effect.

1122-167 Angiotensin II Induces TRAF-2 in Human Vascular Smooth Muscle Cells and Sensitizes CD40 Without Upregulation of CD40 Expression

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Background:
Chronic inflammation of the vessel wall is a hallmark of atherosclerosis. Besides secreted cytokines direct cell-cell contact e.g. via the CD40/CD154 receptor-ligand-dyad contributes to this inflammatory reaction. CD40 is expressed on human vascular smooth muscle cells (SMC) and CD154 on lymphocytes. Intracellular CD40 signaling depends on TNF-receptor associated factors (TRAFs). Aim of the present study was to investigate if the proatherogenic vasoactive peptide angiotensin II (ANG II) stimulates the inflammatory response in human vascular smooth muscle cells via a CD40/CD154-dependent pathway.
Methods and Results:
Human SMC were preincubated with ANG II (100 nM) and afterwards stimulated by recombinant CD154 (5 ng/ml). These cells secreted higher amounts of IL-6 into the culture medium than control cells not primed with ANG II (271 +/- 13 vs. 158 +/-14 pg/ml, $p < 0.05$). This stimulation of cytokine secretion was not due to increased expression of CD40 protein on the cell surface as shown in Western blots but was associated with higher expression of TRAF-2 protein. The effect could be blocked by the specific AT1-receptor antagonists losartan (10 μM) and candesartan (100 nM). Moreover, ANG II time-dependently activated the proinflammatory transcription factors NF-κB and AP-1, as shown in electrophoretic mobility shift assays. The specific proteasome inhibitor PI-1 (50 μM) prevented ANG II-induced TRAF-2 expression showing dependence on NF-κB activation. The antioxidans N-acetyl cysteine (10 mM) inhibited ANG II-induced TRAF-2 expression, too, suggesting involvement of reactive oxygen species in intracellular signaling.
Conclusion:
ANG II induces a functionally relevant inflammatory response in human SMC by induction of TRAF-2. This mechanism may contribute to the proatherogenic effect of ANG II.

1122-168 Urotensin II Modulates Collagen Synthesis and the Expression of Matrix Metallproteinase-1 and Nitric Oxide Synthase in Endothelial Cells

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Background and Objectives: Urotensin II, a potent vasoconstrictor, is a cyclic peptide, which is highly expressed in cardiac and arterial tissues. Recent studies indicate that urotensin II participates in remodeling of myocardium after ischemia. This study was designed to study the role of urotensin II in the expression of matrix matelloproteinase-1 (MMP1), nitric oxide synthases and collagen synthesis in human umbilical artery endothelial cells (HUAECs) and the underlying intracellular signaling mechanism.
Methods and Results: Cultured HUAECs were incubated with urotensin II (10 to 80 nM) for 3 to 24 hours. Urotensin II decreased the expression of MMP1 and increased the expression of collagen in a concentration- and time-dependent fashion. The downregulation of MMP1 expression in response to urotensin II paralleled the increase in collagen synthesis. In addition, urotensin II also changed endothelial function by modestly decreasing constitutive nitric oxide synthase (cNOS) and significantly increasing inducible NOS at 10 and 20 nM. In this process, urotensin II enhanced the activation of mitogen-activated protein kinase (MAPK p42/44), but not MAPK p38. The role of MAPK p42/44 became evident in experiments wherein the pretreatment of cells with the MAPK p42/44 inhibitor (PD98059, 10 μM) inhibited MAPK activation and subsequently attenuated the effects of urotensin II ($P<0.01$ vs. urotensin II alone).
Conclusions:
Our observations provide initial evidence that urotensin II modulates the expression MMP1 and NOS and increases collagen synthesis, and MAPK activation plays a signal transduction role in this process. These findings indicate that urotensin II may play a critical role in remodeling of vascular injury and atherosclerotic plaque.

1122-169 Statins Block Endotoxin-Induced Proinflammatory Responses in Human Coronary Artery Cells by Inhibition of Rho Protein-Mediated Signaling

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Background: Atherosclerosis is increasingly recognized as a chronic inflammatory disorder, and low level endotoxemia has been identified as a powerful risk factor for atherosclerosis.There is also increasing evidence that statins have important anti-inflammatory effects in addition to their lipid-lowering properties. Accordingly, we studied the effects of lovastatin on endotoxin-induced pro-inflammatory responses in human coronary artery endothelial cells (HCAEC) and smooth muscle cells (HCASMC). **Methods:** Interleukin-8 (IL-8) release was measured by ELISA, using matched antibodies. Rho proteins were detected using western blotting. **Results:** Endotoxin-induced IL-8 release was inhibited by lovastatin in a concentration-dependent manner in both cell types. Lovastatin consistently inhibited signaling by endotoxin but not Tumor Necrosis Factor-a (TNF-a), suggesting an effect on a proximal protein in the endotoxin signaling pathway. The inhibition was reversed by mevalonate but not squalene, suggesting involvement of an isoprenylated protein. Endotoxin-induced IL-8 release was inhibited by a specific geranylgeranyl transferase inhibitor but not a farnesyl transferase inhibitor, suggesting an effect on a geranylgeranylated protein, most likely a member of the Rho GTPase family. *Clostridium difficile* toxin B, which glucosylates and inactivates Rho proteins, caused a dose-dependent inhibition of endotoxin-induced IL-8 release; no evidence of cytotoxicity was seen at these concentrations. Endotoxin decreased total HCAEC Rho A and Rho B in a time-dependent manner. Lovastatin increased the total intracellular amount of these proteins, and mevalonate reversed this effect. **Conclusion:** These results suggest that statins may prevent cardiovascular disease in part by direct inhibition of Rho protein-mediated pro-inflammatory activation of coronary artery